ACS NANO

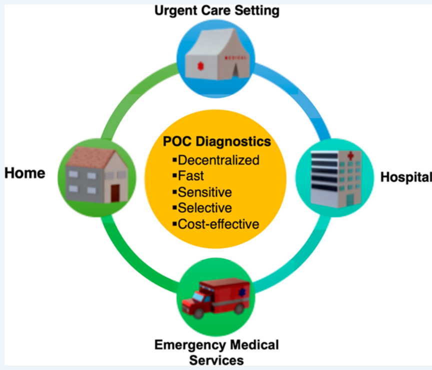
www.acsnano.org

COVID-19 Point-of-Care Diagnostics: Present and Future

Enrique Valera,* Aaron Jankelow, Jongwon Lim, Victoria Kindratenko, Anurup Ganguli, Karen White, James Kumar, and Rashid Bashir*

Cite This: https://doi.org/10.1021/acsnano.1c02981

ABSTRACT: **Point-of-care (POC) detection technologies that enable decentralized, rapid, sensitive, low-cost diagnostics of COVID-19 infection are urgently needed around the world. With many technologies approved for commercialization in the past 10 months, the field of COVID-19 POC diagnostics is rapidly evolving. In this Perspective, we analyze the current state of POC technologies for the diagnosis and monitoring of COVID-19 infection and discuss future challenges in COVID-19 diagnostics. As the COVID-19 pandemic becomes endemic, the advances gained during this past year will likely also be utilized for future prediction of emerging outbreaks and pandemics.**



Since the severe acute respiratory syndrome (SARS-CoV-2) jumped from an animal reservoir to humans in December 2019, it has rapidly spread across the world, bringing critical challenges for public health, including being the number one cause of death in the United States in early 2021, disruption to daily life, and economic losses to businesses and individuals.[1−3] The COVID-19 pandemic has highlighted the need to diagnose the disease rapidly and accurately at scales larger than ever before.[4] Models have predicted that millions of tests per day are needed to remobilize the economy fully.[4,5] However, many factors have contributed to a less-than-optimal availability of testing, including the shortage of laboratory supplies (which also impacts non-COVID testing) and test kits and the inability to scale the supply chain to meet demand.[6,7] Although the current gold standard diagnostic method for the detection of COVID-19 is reverse transcription polymerase chain reaction (RT-PCR) for the RNA of SARS-CoV-2,[8−10] loop-mediated isothermal amplification (LAMP) processes (*e.g.*, reverse-transcription LAMP, RT-LAMP) are also gaining attention.[11−15] Many specimens are approved for use in nucleic acid amplification tests, with the most common being nasopharyngeal (NP), nasal midturbinate, anterior nares, and saliva.[16] It should be noted that swab-based samples are placed in a liquid transport medium, which is then subsequently analyzed.

The development of rapid, point-of-care (POC) molecular diagnostic tests that have sensitivity and specificity comparable to the current gold standard techniques can significantly aid testing expansion.[6,17] Such POC devices could enable the convenient acquisition of information about both viral presence and host response (*e.g.*, antibodies) in nonlaboratory settings with rapid turnaround times. The deployment of testing solutions out of centralized laboratories, for instance, at the primary or urgent care level, could be a key step for the rapid detection and identification of COVID-19 and prevention of transmission to the community.[6] Point-of-care devices offer the possibility of (i) using more portable and cost-effective instrumentation; (ii) eliminating sample transport to a clinical laboratory for analysis; (iii) reducing sample processing; (iv) using samples, such as saliva or anterior nasal swabs, that do not require trained personnel for collection; and (v) measuring different entities (virus, antigen, antibodies) in symptomatic or asymptomatic patients that could contribute to precise determination of individuals who would benefit from clinical care or would require quarantine.

The type of diagnostic solutions needed depend on the throughput, portability, cost, and barriers to regulatory approvals. The testing solutions could be deployed out of

https://doi.org/10.1021/acsnano.1c02981
ACS Nano XXXX, XXX, XXX−XXX

The development of rapid, point-of-care molecular diagnostic tests that have sensitivity and specificity comparable to the current gold standard techniques can significantly aid testing expansion.

centralized laboratories and could offer throughputs ranging from thousands of tests per day to one test for personal use. Figure 1 shows the COVID-19 portable diagnostics options. The size of the diagnostics solution is typically inversely proportional to the portability and is directly proportional to the testing capacity. Whereas POC technologies are considered for self-use using hand-held devices, other factors for portable approaches such as a mobile laboratory, a self-contained benchtop system, or a suitcase that can be used for testing in large, medium, or small gatherings of people, respectively, should also be considered.

Point-of-care approaches are those that can provide results at the point of use, such as at home, or in hospitals, urgent care centers, elderly care centers, emergency rooms, or other settings, instead of samples being sent to a laboratory. These tests can still be used under the auspices of a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory or used by an individual for self-testing. The tests may require a trained individual to collect the sample and to perform the analysis or they could also be used for self-testing by the patient themselves. Receiving a result should be as rapid as possible and not limited by the assay itself, but by the data and information management system used by the company or hospital providing the assay.

The United States used Emergency Use Authorization (EUA) to enable emergency use of *in vitro* diagnostics for detection of SARS-CoV-2 or diagnosis of COVID-19 to expedite the process of such devices entering the commercial market.[18] After EUA authorization, the test is categorized and can be performed in a particular setting under CLIA (*e.g.*, moderate complexity or POC). This policy does not apply to at-home testing.[19] In addition, EUA requests for COVID-19 diagnostic tests that can be performed entirely at home or in other settings outside a lab have their own recommendations concerning what data and information should be submitted with the request.[20] For instance, due to the greater potential for error in specimen collection at home, the FDA recommends that the device has an internal control to indicate that an adequate human sample was collected and placed into the test for analysis. Likewise, the use of anterior nares (nasal) swabs, midturbinate swabs, or saliva as sample types is recommended to avoid the use of incorrect techniques that could result in patient harm.

When a subject exhibits signs of COVID-19, physicians need to test for the presence of COVID-19 and to quantify the severity of the disease, which can range from mild to critical. Symptomatic patients are isolated while awaiting test results. Highly suspect cases may remain isolated based on clinician judgment until follow up confirmatory testing through either repeat molecular methods or serology. Based on the illness severity and comorbidity conditions, physicians need to decide if the subject will require guideline-directed therapeutic management in the appropriate setting. Early and accurate testing is necessary to help guide the decision as to which clinical path the patient might follow. Consequences of these delays in hospitals include poor patient flow and possible nosocomial transmission.[21] Therefore, rapid and accurate POC tests that can detect acute or past SARS-CoV-2 infections and that do not rely on centralized laboratories are urgently needed to lighten the demand for tests in hospitals and to ensure faster results to the population.

WHAT SHOULD BE DETECTED?

Currently, there are three types of COVID-19 tests: molecular diagnostics, antigen tests, and antibody tests (Table 1). Molecular diagnostics tests indicate the presence of the SARS-CoV-2 RNA, antigen tests detect specific proteins from the virus, and antibody tests determine whether the individual has developed antibodies to the virus.[22,23] For molecular tests, the available targets are different regions of the RNA genome, whereas for antigen tests, the targets are the available structural proteins (antigens) that are anchored on or inside the viral envelop (Figure 2). SARS-CoV-2 virus contains a single-stranded RNA that includes target genes such as ORF1b, ORF8, N-protein, S-protein, RNA-dependent RNA polymerase, and envelope genes.[24] The four major structural proteins are the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N).[25]

MOLECULAR, ANTIGEN, AND ANTIBODY TEST POINT-OF-CARE DEVICES

The high specificity of RT-PCR and its ability to make billions of copies of a specific RNA or DNA sample rapidly make this amplification method the current gold standard for the detection of the SARS-CoV-2 virus. However, the reliance on thermal

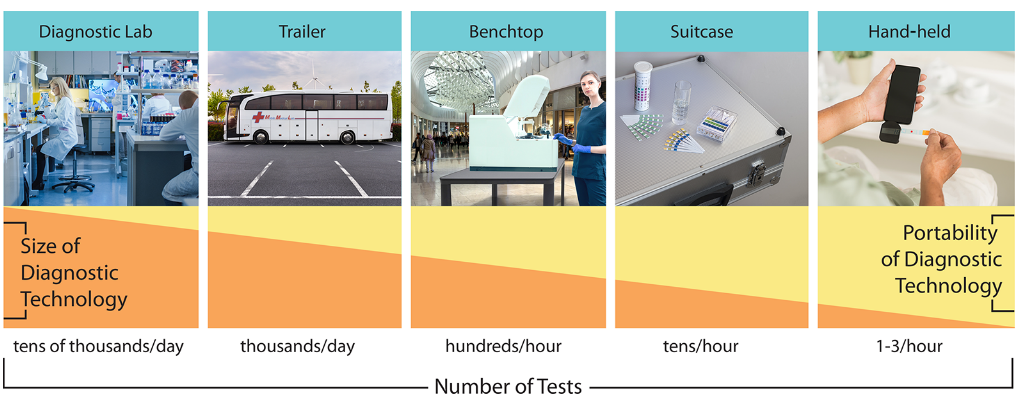


Figure 1. Our vision for COVID-19 portable diagnostics options.

https://doi.org/10.1021/acsnano.1c02981
ACS Nano XXXX, XXX, XXX−XXX

Table 1. COVID-19 Tests: Relevant Features[a]

COVID-19 Tests	Target	Outcome	Specimen Used	Time of Assay	Time to Result	Comments
Molecular	Viral RNA (N, E, S, ORF1ab, ORF3a, and ORF7ab, genes)	Detects presence of RNA in the sample, implying an active infection in the individual (whether symptomatic or asymptomatic)	• NP swab • Nasal swab • Throat swab • Saliva	< 2 h	Hours to days	• Most sensitive, as target is exponentially amplified before it is detected. • More challenging to implement outside the laboratory environment. • Long turnaround for time to result due to inability to scale the laboratory tests.
Antigen	Viral antigens (N-protein and S-protein)	Presence of viral proteins and fragments or whole virus, implying an active coronavirus infection in the individual (whether symptomatic or asymptomatic)	• NP swab • Nasal swab • Saliva	15–30 min	Hours to days	• Less sensitive, as target is not amplified. • Higher false-negative rate (If subject has symptoms and the test is negative, a molecular test may be needed for confirmation). • Easier to implement outside the laboratory environment. • Time to result limited by the laboratory's data and information management system.
Antibody	IgM and IgG antibodies	Detection of Abs produced by the body indicating resolving or past SARS-CoV-2 virus infection	• Finger stick • Blood draw	15–30 min	Hours to days	• IgM and IgG antibodies may take 1 to 3 weeks to develop after infection • The presence of antibodies cannot be equated with an individual's immunity.[37,38] • Some tests may exhibit cross-reactivity with other coronaviruses.[40] • Some persons may not develop detectable antibodies after coronavirus infection, or antibody levels may wane over time to undetectable levels.[40] • Time to result limited by the laboratory's data and information management system.

[a]Based on refs 22 and 40. NP = Nasopharyngeal.

cycling makes it difficult to translate this technology to a portable device due to the variance and accuracy in temperatures needed to amplify the genetic material in the sample. Likewise, the standard RT-PCR protocol utilizes an RNA extraction and purification step using commercially available kits. The RNA extraction kit not only extracts the RNA from the virus but also purifies the RNA and may also concentrate it depending on the volume of fluid used after purification, hence contributing to improving assay sensitivity.

Successful examples of EUA-approved RT-PCR-based POC devices are the Xpert Xpress SARS-CoV-2, Xpert Xpress SARS-CoV-2/Flu/RSV, Xpert Xpress SARS-CoV-2 DoD (all three from Cepheid), Accula SARS-CoV-2 Test (Mesa Biotech Inc.), cobas SARS-CoV-2 and Influenza A/B Nucleic Acid Test (Roche Molecular Systems, Inc.), BioFire Respiratory Panel 2.1-EZ (BioFire Diagnostics, LLC), and Visby Medical COVID-19 Point-of-Care Test (Visby Medical, Inc.).[26] One example, the Visby Medical test, is a single-use (disposable), fully integrated test, where anterior nasal or midturbinate swabs samples can be self-collected by individuals 18 years of age or older, under the supervision of a health care provider.[27] All of these EUA-approved tests are authorized for use at the POC (*i.e.*, in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation).

Isothermal amplification-based approaches have recently generated significant attention for detection of SARS-CoV-2 virus due to the simplicity of this technology (typically one-step) and the ease of translation to a point-of-use device, as these technologies eliminate the need for precise thermal cycles to achieve RNA amplification.[11,12] Additional advantages are the possible elimination of the viral purification step, and the simplification of the instrumentation complexity.[11,12] Shortly after the pandemic started, Abbott Diagnostics Scarborough, Inc. released the ID NOW COVID-19 test which uses RT-LAMP. This was the first isothermal technology to receive EUA authorization for COVID-19 testing.[26] Cue COVID-19 Test (Cue Health, EUA approved) utilizes isothermal amplification (20 min) in a single-use cartridge that detects the virus from direct nasal swabs with a limit of detection of 20 genome copies per sample using an electrochemical detection method.[14] The reader, which is not included in the test cartridge pack, can run thousands of tests before it needs to be replaced.[28] Similar to the EUA-approved PCR devices, ID NOW COVID-19 and Cue COVID-19 Test are authorized for use at the POC (*i.e.*, in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation). On March 5, 2021, Cue Health took an important step when the Cue COVID-19 Test for home and over the counter (OTC) use was approved for nonprescription home use, thus becoming the nation's first molecular diagnostic test available without a prescription to consumers for home use and to enterprise users and healthcare professionals without CLIA certification.[14] Another example of a single-use test is the Lucira COVID-19 All-in-One Test Kit (Lucira Health, Inc.). This rapid

https://doi.org/10.1021/acsnano.1c02981
ACS Nano XXXX, XXX, XXX−XXX

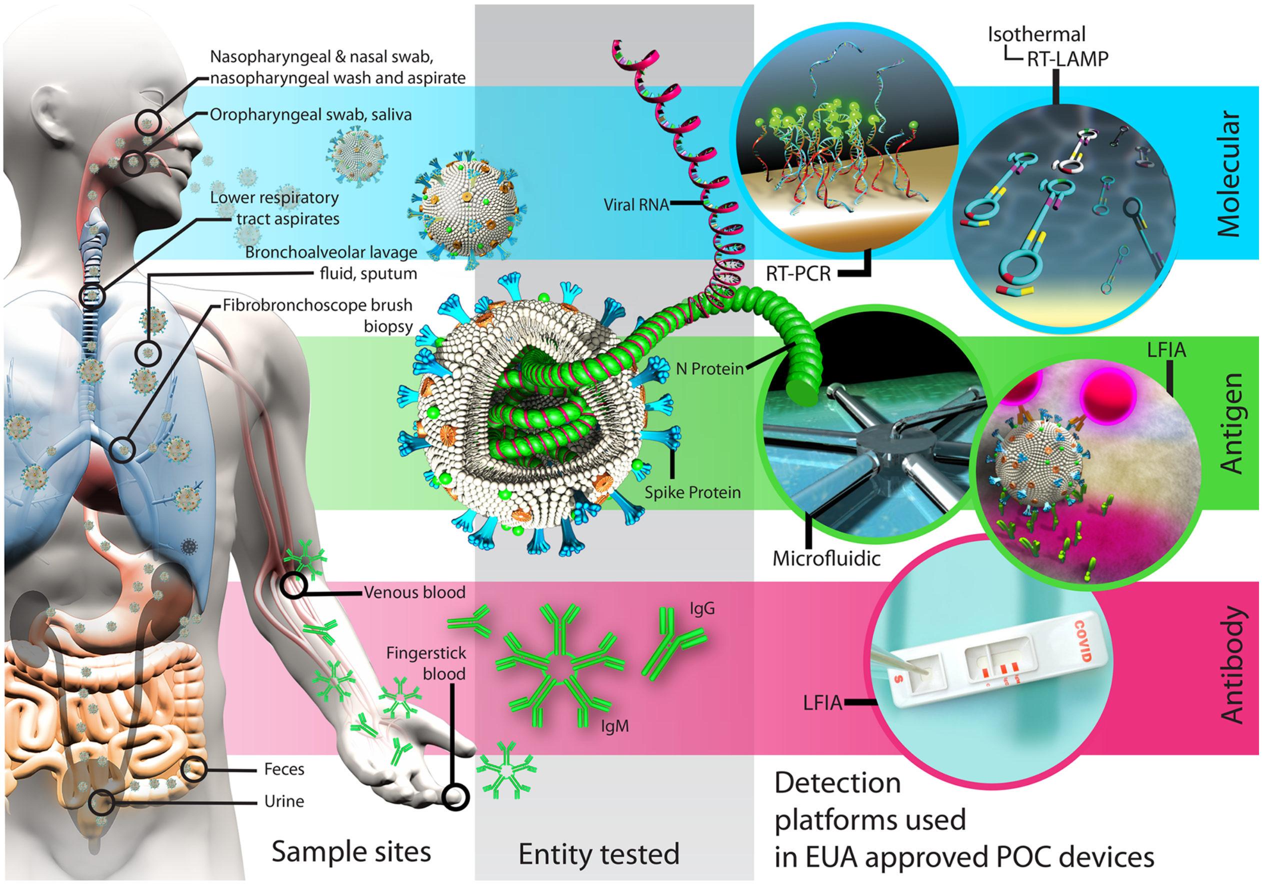


Figure 2. Summary of human samples where the SARS-CoV-2 virus can be found, targets that can be tested, and detection platforms used in Emergency Use Authorization (EUA)-approved point-of-care (POC) devices. Blue row connects viral RNA with sample source and molecular amplification technologies. Green row connects viral proteins with sample source and technologies used for antigen tests. Red row connects antibodies with blood sources and technologies used for antibody tests. RT-PCR, reverse-transcription polymerase chain reaction; RT-LAMP, reverse-transcription loop-mediated isothermal amplification; and LFIA, lateral flow immunoassay.

(30 min) RT-LAMP-based hand-held battery-powered device, which received the first FDA authorization for COVID-19 self-testing at home, enables individuals 14 years and older to test themselves using self-collected nasal swabs.[15]

> Isothermal amplification-based approaches have recently generated significant attention for detection of the SARS-CoV-2 virus due to the simplicity of this technology (typically one-step) and the ease of translation to a point-of-use device.

Antigen tests rely on specific monoclonal antibodies to detect the SARS-CoV-2 structural proteins. These tests have been highlighted as a potentially important tool in an overall community testing strategy to reduce transmission.[29] Although most of the currently available antigen tests target the N-protein, the use of the S-protein may be more specific because this protein has less sequence homology with the previous SARS-CoV and MERS viruses.[30] Antigen tests are faster than PCR techniques (providing results in a few minutes); however, they are inherently less sensitive as no amplification of the target is involved. Likewise, these tests provide qualitative results only (they do not quantify the viral load in the sample). As seen in Table 1, antigen devices also have high false-negative rates. Thus, a negative test result may occur if the level of antigen in a sample is below the detection limit of the test.

Successful examples of antigen EUA-approved POC devices are the LumiraDx SARS-CoV-2 Ag Test (LumiraDx UK Ltd.), CareStart COVID-19 Antigen test (Access Bio, Inc.), BinaxNOW COVID-19 Ag Card (Abbott Diagnostics Scarborough, Inc.), BD Veritor System for Rapid Detection of SARS-CoV-2 (Becton, Dickinson and Company, LLC), Clip COVID Rapid Antigen Test (Luminostics, Inc.), QuickVue SARS Antigen Test, Sofia 2 SARS Antigen FIA, Sofia 2 Flu + SARS Antigen FIA (all three from Quidel Corporation), and *Status* COVID-19/Flu (Princeton BioMeditech Corp.). All of these technologies provide qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2. Therefore, all of them include an extraction buffer to disrupt the virus particles present in the specimen and expose the internal viral nucleoproteins.[26] Likewise, all of these EUA-approved technologies are authorized for use at the POC (*i.e.*, in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation) and require trained operators.

In December 2020, the Ellume COVID-19 Home Test (Ellume Limited, EUA approved) became the first antigen test to be authorized for nonprescription, OTC home use.[31] The

https://doi.org/10.1021/acsnano.1c02981
ACS Nano XXXX, XXX, XXX−XXX

Ellume test is not yet available for purchase, however the estimated cost is \$30. Another antigen home test is the BinaxNOW COVID-19 Ag Card Home Test (Abbott Diagnostics Scarborough, Inc., EUA approved).[32] Unlike the Ellume device, this test requires a prescription and is to be performed only under the supervision of a telehealth proctor. On March 31, 2021, tests from the BinaxNOW family were authorized for nonprescription home use with self-collected samples from individuals aged 15 years and older or adult-collected anterior nasal swab samples from individuals aged 2 plus years old (BinaxNOW COVID-19 Antigen Self-Test[33] and BinaxNOW COVID-19 Ag Card 2 Home Test).[34] The options of at-home tests have also been expanded with the new members of the QuickVue family, the QuickVue At-Home OTC COVID-19 Test[35] and the QuickVue At-Home COVID-19 Test.[36] These devices clearly indicate a trend in the antigen testing market focusing on at-home testing.

The detection of antibodies to the SARS-CoV-2 virus cannot be considered an "immunity passport" or "risk-free certificate". It is currently unknown if people who have recovered from COVID-19 and have antibodies are protected from being infected again, because some confirmed and suspected cases of reinfection have been reported.[37,38] Likewise, depending on the timing of infection and sampling for serologic testing, recently infected individuals may be antibody positive while still shedding the virus.[39] However, important roles such as determining the true prevalence of this virus and monitoring the temporal immune responses in vaccine recipients are expected to be accomplished by serologic testing.[39]

Although more than 100 serology tests have been EUA approved (including EUA submission pending) in recent months, only a few of them have been approved as POC devices: Assure COVID-19 IgG/IgM Rapid Test Device (Assure Tech.), RightSign COVID-19 IgG/IgM Rapid Test Cassette (Hangzhou Biotest Biotech), RapCov Rapid COVID-19 Test (Advaite, Inc.), MidaSpot COVID-19 Antibody Combo Detection Kit (Nirmidas Biotech, Inc.), and Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test Cassette (Salofa Oy).[26] Similar to the EUA-approved molecular and antigen devices, EUA-approved antibody tests are authorized for use at the POC (*i.e.*, in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation). Most of these devices provide qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human venous whole blood, serum, and plasma, or finger-stick whole blood. All these tests are rapid (10−20 min) and include control lines.

CURRENT AND FUTURE CHALLENGES

One of the major challenges during 2020 was the development and scaling of reliable methods for SARS-CoV-2 molecular detection and serologic assays. Although diagnostic testing for COVID-19 is critical to controlling the spread of the virus by quarantining,[41] mass testing needs to be strategically deployed so as not to prevent access to limited testing resources by those who need them most[10] and not affect other routine microbial tests for a wide range of infectious diseases due to supply chain limitations.[42] The same will be true for the vaccination and postvaccination eras, where diagnostic and serology POC devices could strategically improve COVID-19 care, reduce costs and supply chain restrictions, and increase our understanding of the underlying mechanisms of pathogenicity, infectivity, and immunity detection. Diagnostics will remain important because even vaccinated patients, while asymptomatic, may still carry and transmit live virus from the upper airway.[43] Some specific points are expanded below.

- Molecular diagnostics tests such as RT-PCR are the gold standard for detecting the presence of SARS-CoV-2 virus due to high sensitivity and reliability. However, these processes still take longer than desired. RT-PCR tests can take 60−90 min minimum plus the time for data analysis and reporting. Although isothermal amplification techniques such as RT-LAMP can reduce this time, the time for the assay is 30−45 min plus data reporting to provide the final results to the patient. On the other hand, antigen tests are rapid and can provide results in as little as 5 min but are ∼100× less sensitive. Therefore, one of the main goals for POC devices is the development of a molecular amplification-based test that can provide results in 5 min at the cost of an antigen test. Such a device would replace antigen tests while offering high sensitivity.
- It is expected that COVID-19 will become an endemic disease.[44] Therefore, multiplexed POC devices that are able to test and to differentiate between different coronavirus variants and other seasonal respiratory illnesses, such as the flu, will be needed. The performance of these devices should be tested across all known variants at the time of validation while taking into account the potential impact of future variants, as recommended by the FDA.[45]
- Routes to reduce the overall cost of sample collection, testing, and analysis are necessary. In this direction, the use of saliva as a specimen could be a satisfactory solution if the sensitivity of the assay is not affected. Saliva has demonstrated to be an alternative upper respiratory tract specimen type for SARS-CoV-2 detection.[12,46,47] Likewise, saliva offers a number of advantages over nasopharyngeal swabs when considering mass testing, as it can be self-administered. For instance, it is known that the use of NP swabs can cause discomfort or irritation and can increase the risk of exposure for the medical providers;[48,49] variation in nasopharyngeal sampling may be an explanation for false negative results.[46] In contrast, saliva collection does not require a certified swab, specific collection receptacle, or transport media and does not have to be obtained by a skilled healthcare provider.[12] Importantly, RNA purification-free RT-PCR and RT-LAMP assays have been developed for detection of SARS-CoV-2 from saliva clinical samples.[12,47]
- Importantly, it should also be noted that clinical testing practices are migrating to nasal swabs from anterior nostrils, which can also be self-administered. We believe that the use of either nasal swabs of the anterior nostrils or saliva will facilitate the scaling of diagnostics. The sensitivity and specificity of the tests from these sources can be different and saliva can be expected to be a more sensitive indicator of the state of the respiratory system.
- Increasing testing capacity and increasing antigen and molecular testing manufacturing in the United States are part of President Biden's National Strategy for the COVID-19 Response and Pandemic Preparedness.[50] In particular, there is a major interest in OTC, at-home testing, and we think this is one of the most attractive directions. For instance, Ellume USA was recently awarded \$231.8 million to produce the Ellume COVID-

https://doi.org/10.1021/acsnano.1c02981
ACS Nano XXXX, XXX, XXX−XXX

19 Home Test.[51] Likewise, the Rapid Acceleration of Diagnostics (RADx) initiative plans to invest $1.5 billion to speed development of rapid and widely accessible COVID-19 testing,[52] including recent announcements of over $250 million to increase the testing capacity at the POC or at home.[51,53]

- With new molecular and antigen devices authorized for OTC, at-home testing, the challenges will be to ensure adequate sample collection (to ensure the quality of the test), correct collection technique (to avoid patient harm), and a price that allows continuous access to available tests.
- As we learn more and advance our understanding of diagnostics, it is important to note that assessing the presence of infectious viruses is the ultimate information desired. The presence of viral RNA or antigens currently is assumed to correlate with the presence of infectious virus. For instance, a nasal swab can pick up inactivated viruses or RNA only and, hence, someone could test positive while not being infectious or even sick (false positive for disease). To prove the presence of infectious virus or to examine the infectivity of the virus, propagation of viruses in a cell line (plaque assays) is usually performed.[54] Microfluidic devices for developing cells, tissues, and organoid models, similar to what is being done for cancer drug screening, can be used as organ mimics on a chip to test for the presence of infectious viruses in a sample.
- Understanding the biological mechanisms of COVID-19 is crucial in navigating our responses including effective therapeutic and preventative measures.[1] SARS-CoV-2 pathogenesis is complex: For instance, some virus variants can be associated with higher viral loads in COVID-19 patients but not with disease severity.[55] More testing is required to understand the routes of transmission of the virus, such as fecal–oral, and to understand whether patients recovering from COVID-19-related respiratory illnesses are able to spread SARS-CoV-2.[56] As discussed above, the study of pathogenesis can be aided by the development of engineered microfluidic organ-on-chip platforms and cell-based models. These technologies can be useful for testing drugs as potential therapeutics against SARS-CoV-2 or for screening and precisely analyzing molecular pathways of COVID-19 pathogenesis.[57,58]
- The duration for which immunity lasts after infection or after vaccination is not yet known,[59] and, hence, the rapid and reliable detection of immunity after an individual gets COVID-19 or receives the vaccination will be crucial. Gingras *et al.* recently reported that anti-SARS-CoV-2 antibodies were detected in serum and saliva, with peak IgG levels attained by 16–30 days postsymptom onset. Their study revealed that anti-SARS-CoV-2 IgA and IgM antibodies rapidly decayed, whereas IgG antibodies remained stable up to 105 days in both biofluids.[60] Studies have reported that IgG can suggest immunity; however, in some cases, the sensitivity can be as low as 70.5% for the lateral flow devices used to detect antibodies.[61]
- Many reports have demonstrated that COVID-19 disproportionately impacted people of color and under-resourced regions.[62,63] Access to testing was limited in these communities and exacerbated the impact of the pandemic. Use of POC devices can significantly aid in bridging the social divide in COVID-19 and other pandemics in the future.
- New approaches are needed to capture virus particles in their aerosolized forms. Self-contained systems that can reliably sample and capture particles from air, and subsequently identify the viruses using RNA amplification or detection of antigens, would have widespread use.
- Finally, we note that POC device technologies should be ready for any future outbreaks, from new strains or novel viruses. The response to this pandemic when it comes to drug development has been extremely fast. However, the need for a diagnostic upscale was not met. The POC technology platforms developed this year should quickly be translated to the detection of new pathogens, once the sequence of the new pathogens is known.

AUTHOR INFORMATION

Corresponding Authors

Enrique Valera – *Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States;* orcid.org/0000-0003-1359-6619; Email: evalerac@illinois.edu

Rashid Bashir – *Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States; Department of Biomedical and Translational Science, Carle Illinois College of Medicine, Urbana, Illinois 61801, United States;* orcid.org/0000-0002-7225-9180; Email: rbashir@illinois.edu

Authors

Aaron Jankelow – *Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States*

Jongwon Lim – *Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States*

Victoria Kindratenko – *Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States*

Anurup Ganguli – *Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States*

Karen White – *Department of Biomedical and Translational Science, Carle Illinois College of Medicine, Urbana, Illinois 61801, United States; Carle Foundation Hospital, Urbana, Illinois 61801, United States*

James Kumar – *Department of Biomedical and Translational Science, Carle Illinois College of Medicine, Urbana, Illinois 61801, United States; Carle Foundation Hospital, Urbana, Illinois 61801, United States*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsnano.1c02981

Notes

The authors declare no competing financial interest.

https://doi.org/10.1021/acsnano.1c02981
ACS Nano XXXX, XXX, XXX–XXX

ACKNOWLEDGMENTS

R.B. and E.V. acknowledge support from the Foxconn Interconnect Technology sponsored Center for Networked Intelligent Components and Environments (C-NICE) and the Jump Applied Research through Community Health through Engineering and Simulation (ARCHES) endowment through the Health Care Engineering Systems Center at the University of Illinois at Urbana−Champaign. We also thank the National Science Foundation for a Rapid Response Research (RAPID) grant (award 2028431) to R.B. and E.V.

REFERENCES

(1) Fauci, A. S.; Lane, H. C.; Redfield, R. R. COVID-19 — Navigating the Uncharted. *N. Engl. J. Med.* **2020**, *382*, 1268−1269.

(2) Nicola, M.; Alsafi, Z.; Sohrabi, C.; Kerwan, A.; Al-Jabir, A.; Iosifidis, C.; Agha, M.; Agha, R. The Socio-Economic Implications of the Coronavirus Pandemic (COVID-19): A Review. *Int. J. Surg.* **2020**, *78*, 185−193.

(3) Cox, C.; Amin, K. *COVID-19 is the Number One Cause of Death in the U.S. in Early 2021*. https://www.healthsystemtracker.org/brief/covid-19-is-the-number-one-cause-of-death-in-the-u-s-in-early-2021/ (accessed 2021-02-17).

(4) Allen, D.; Block, S.; Cohen, J.; Eckersley, P.; Eifler, M.; Gostin, L.; Goux, D.; Gruener, D.; Hart, V.; Hitzig, Z.; Krein, J.; Langford, J.; Nordhaus, T.; Rosenthal, M.; Sethi, R.; Siddarth, D.; Simons, J.; Sitaraman, G.; Slaughter, A.-M.; Stanger, A.; et al. *Roadmap to Pandemic Resilience - Massive Scale Testing, Tracing, and Supported Isolation (TTSI) as the Path to Pandemic Resilience for a Free Society*. https://ethics.harvard.edu/files/center-for-ethics/files/roadmaptopandemicresilience_updated_4.20.20_0.pdf (accessed 2021-02-05).

(5) Tromberg, B. J.; Schwetz, T. A.; Pérez-Stable, E. J.; Hodes, R. J.; Woychik, R. P.; Bright, R. A.; Fleurence, R. L.; Collins, F. S. Rapid Scaling Up of COVID-19 Diagnostic Testing in the United States — The NIH RADx Initiative. *N. Engl. J. Med.* **2020**, *383*, 1071−1077.

(6) Vandenberg, O.; Martiny, D.; Rochas, O.; van Belkum, A.; Kozlakidis, Z. Considerations for Diagnostic COVID-19 Tests. *Nat. Rev. Microbiol.* **2021**, *19*, 171−183.

(7) American Society for Microbiology. *Laboratory Supply Shortages Are Impacting COVID-19 and Non-COVID Diagnostic Testing*. https://asm.org/Articles/2020/September/Laboratory-Supply-Shortages-Are-Impacting-COVID-19 (accessed 2021-02-05).

(8) Centers for Disease Control and Prevention. *Interim Guidance for Antigen Testing for SARS-CoV-2*. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html (accessed 2020-12-11).

(9) Wang, R.; Qian, C.; Pang, Y.; Li, M.; Yang, Y.; Ma, H.; Zhao, M.; Qian, F.; Yu, H.; Liu, Z.; Ni, T.; Zheng, Y.; Wang, Y. opvCRISPR: One-Pot Visual RT-LAMP-CRISPR Platform for SARS-CoV-2 Detection. *Biosens. Bioelectron.* **2021**, *172*, 112766.

(10) Binnicker, M. J. Challenges and Controversies to Testing for COVID-19. *J. Clin. Microbiol.* **2020**, *58*, e01695−20.

(11) Ganguli, A.; Mostafa, A.; Berger, J.; Aydin, M. Y.; Sun, F.; Ramirez, S. A. S. d.; Valera, E.; Cunningham, B. T.; King, W. P.; Bashir, R. Rapid Isothermal Amplification and Portable Detection System for SARS-CoV-2. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117*, 22727−22735.

(12) Ganguli, A.; Mostafa, A.; Berger, J.; de Ramirez, S. A. S.; Baltaji, A.; Roth, K.; Aamir, M.; Aedma, S.; Mady, M.; Mahajan, P.; Sathe, S.; Johnson, M.; White, K.; Kumar, J.; Valera, E.; Bashir, R. RT-LAMP Assay for Ultra-Sensitive Detection of SARS-CoV-2 in Saliva and VTM Clinical Samples. *medRxiv*, November 18, 2020, ver. 1. DOI: 10.1101/2020.11.16.20232678 (accessed Dec 16, 2020).

(13) *ID NOW COVID-19*. https://www.globalpointofcare.abbott/en/product-details/id-now-covid-19.html (accessed 2020-12-16).

(14) *Cue Health Leading the fight against COVID-19*. https://www.cuehealth.com/what-is-cue/how-cue-detects-covid-19 (accessed 2020-12-19).

(15) *Lucira Check It*. https://www.lucirahealth.com/ (accessed April 6, 2021).

(16) Centers for Disease Control and Prevention. *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19*. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html#specimen (accessed 2021-02-05).

(17) van Dongen, J. E.; Berendsen, J. T.W.; Steenbergen, R. D.M.; Wolthuis, R. M.F.; Eijkel, J. C.T.; Segerink, L. I. Point-of-Care CRISPR/Cas Nucleic Acid Detection: Recent Advances, Challenges and Opportunities. *Biosens. Bioelectron.* **2020**, *166*, 112445.

(18) U.S. Food & Drug Administration. *Emergency Use Authorization of Medical Products and Related Authorities*. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/emergency-use-authorization-medical-products-and-related-authorities (accessed 2021-01-09).

(19) U.S. Food & Drug Administration. *Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)*. https://www.fda.gov/media/135659/download (accessed 2021-02-01).

(20) U.S. Food & Drug Administration. *Coronavirus (COVID-19) Update: FDA Posts New Template for At-Home and Over-the-Counter Diagnostic Tests for Use in Non-Lab Settings, Such as Homes, Offices or Schools*. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-posts-new-template-home-and-over-counter-diagnostic-tests-use-non (accessed 2021-03-02).

(21) Brendish, N. J.; Poole, S.; Naidu, V. V.; Mansbridge, C. T.; Norton, N. J.; Wheeler, H.; Presland, L.; Kidd, S.; Cortes, N. J.; Borca, F.; Phan, H.; Babbage, G.; Visseaux, B.; Ewings, S.; Clark, T. W. Clinical Impact of Molecular Point-of-Care Testing for Suspected COVID-19 in Hospital (COV-19POC): A Prospective, Interventional, Non-Randomised, Controlled Study. *Lancet Respir. Med.* **2020**, *8*, 1192−1200.

(22) U.S. Food & Drug Administration. *Coronavirus Disease 2019 Testing Basics*. https://www.fda.gov/consumers/consumer-updates/coronavirus-disease-2019-testing-basics (accessed 2020-12-12).

(23) Kilic, T.; Weissleder, R.; Lee, H. Molecular and Immunological Diagnostic Tests of COVID-19: Current Status and Challenges. *iScience* **2020**, *23*, 101406.

(24) Carter, L. J.; Garner, L. V.; Smoot, J. W.; Li, Y.; Zhou, Q.; Saveson, C. J.; Sasso, J. M.; Gregg, A. C.; Soares, D. J.; Beskid, T. R.; Jervey, S. R.; Liu, C. Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Cent. Sci.* **2020**, *6*, 591−605.

(25) Wu, A.; Peng, Y.; Huang, B.; Ding, X.; Wang, X.; Niu, P.; Meng, J.; Zhu, Z.; Zhang, Z.; Wang, J.; Sheng, J.; Quan, L.; Xia, Z.; Tan, W.; Cheng, G.; Jiang, T. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. *Cell Host Microbe* **2020**, *27*, 325−328.

(26) Genomeweb. *Coronavirus Test Tracker: Commercially Available COVID-19 Diagnostic Tests*. https://www.360dx.com/coronavirus-test-tracker-launched-covid-19-tests (accessed 2020-12-19).

(27) U.S. Food & Drug Administration Emergency Use Authorization. *Visby Medical COVID-19 Point of Care Test*. https://www.fda.gov/media/145914/download (accessed 2021-04-05).

(28) Genomeweb. *Cue Health Ramping Up COVID-19 Test Production Following Positive Mayo Clinic Study, DoD Funding*. https://www.genomeweb.com/molecular-diagnostics/cue-health-ramping-covid-19-test-production-following-positive-mayo-clinic#.YD_KQpNKiL4 (accessed 2021-03-03).

(29) Prince-Guerra, J. L.; Almendares, O.; Nolen, L. D.; Gunn, J. K. L.; Dale, A. P.; Buono, S. A.; Deutsch-Feldman, M.; Suppiah, S.; Hao, L.; Zeng, Y.; Stevens, V. A.; Knipe, K.; Pompey, J.; Atherstone, C.; Bui, D. P.; Powell, T.; Tamin, A.; Harcourt, J. L.; Shewmaker, P. L.; Medrzycki, M.; et al. Evaluation of Abbott BinaxNOW Rapid Antigen Test for SARS-CoV-2 Infection at Two Community-Based Testing Sites — Pima County, Arizona, November 3−17, 2020. *Morb. Mortal. Wkly. Rep.* **2021**, *70*, 100−105.

(30) Mojsoska, B.; Larsen, S.; Olsen, D. A.; Madsen, J. S.; Brandslund, I.; Alatraktchi, F. A. a. Rapid SARS-CoV-2 Detection Using Electrochemical Immunosensor. *Sensors* **2021**, *21*, 390.

(31) National Institutes of Health. *NIH-funded COVID-19 Home Test Is First to Receive Over-the-Counter Authorization from FDA*. https://

www.nih.gov/news-events/news-releases/nih-funded-covid-19-home-test-first-receive-over-counter-authorization-fda (accessed 2021-03-03).

(32) Abbott. *Abbott's BinaxNOW COVID-19 Rapid Test Receives FDA Emergency Use Authorization for First Virtually Guided, At-Home Rapid Test Using Emed's Digital Health Platform*. https://abbott.mediaroom.com/2020-12-16-Abbotts-BinaxNOW-COVID-19-Rapid-Test-Receives-FDA-Emergency-Use-Authorization-for-First-Virtually-Guided-At-Home-Rapid-Test-Using-eMeds-Digital-Health-Platform (accessed 2021-03-02).

(33) U.S. Food & Drug Administration Emergency Use Authorization. *BinaxNOW COVID-19 Antigen Self Test*. https://www.fda.gov/media/147251/download (accessed 2021-04-05).

(34) U.S. Food & Drug Administration Emergency Use Authorization. *BinaxNOW COVID-19 Ag Card 2 Home Test*. https://www.fda.gov/media/147256/download (accessed 2021-04-05).

(35) U.S. Food & Drug Administration Emergency Use Authorization. *QuickVue At-Home OTC COVID-19 Test*. https://www.fda.gov/media/147247/download (accessed 2021-04-05).

(36) U.S. Food & Drug Administration Emergency Use Authorization. *QuickVue At-Home COVID-19 Test*. https://www.fda.gov/media/146309/download (Accessed 2021-04-05).

(37) World Health Organization. *"Immunity passports" in the Context of COVID-19*. https://www.who.int/news-room/commentaries/detail/immunity-passports-in-the-context-of-covid-19 (accessed 2021-05-03).

(38) Centers for Disease Control and Prevention. *Using Antibody Tests for COVID-19*. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests.html. (accessed 2021-01-29).

(39) Theel, E. S.; Slev, P.; Wheeler, S.; Couturier, M. R.; Wong, S. J.; Kadkhoda, K. The Role of Antibody Testing for SARS-CoV-2: Is There One? *J. Clin. Microbiol.* **2020**, *58*, e00797-20 DOI: 10.1128/JCM.00797-20.

(40) Centers for Disease Control and Prevention. *Interim Guidelines for COVID-19 Antibody Testing*. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html (accessed 2020-12-12).

(41) Centers for Disease Control and Prevention. *CDC Guidance for Expanded Screening Testing to Reduce Silent Spread of SARS-CoV-2*. https://www.cdc.gov/coronavirus/2019-ncov/php/open-america/expanded-screening-testing.html (accessed 2021-02-11).

(42) U. S. Government Accountability Office. *COVID-19: Critical Vaccine Distribution, Supply Chain, Program Integrity, and Other Challenges Require Focused Federal Attention*. https://www.gao.gov/products/gao-21-265 (accessed 2021-02-11).

(43) Bleier, B. S.; Ramanathan, M.; Lane, A. P. COVID-19 Vaccines May Not Prevent Nasal SARS-CoV-2 Infection and Asymptomatic Transmission. *Otolaryngol.–Head Neck Surg.* **2021**, *164*, 305−307.

(44) Phillips, N. The Coronavirus Will Become Endemic. *Nature* **2021**, *590*, 382−384.

(45) U.S. Food & Drug Administration. *Policy for Evaluating Impact of Viral Mutations on COVID-19 Tests*. https://www.fda.gov/media/146171/download (accessed 2021-02-23).

(46) Wyllie, A. L.; Fournier, J.; Casanovas-Massana, A.; Campbell, M.; Tokuyama, M.; Vijayakumar, P.; Warren, J. L.; Geng, B.; Muenker, M. C.; Moore, A. J.; Vogels, C. B. F.; Petrone, M. E.; Ott, I. M.; Lu, P.; Venkataraman, A.; Lu-Culligan, A.; Klein, J.; Earnest, R.; Simonov, M.; Datta, R.; et al. Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. *N. Engl. J. Med.* **2020**, *383*, 1283−1286.

(47) Ranoa, D. R. E.; Holland, R. L.; Alnaji, F. G.; Green, K. J.; Wang, L.; Brooke, C. B.; Burke, M. D.; Fan, T. M.; Hergenrother, P. J. Saliva-Based Molecular Testing for SARS-CoV-2 that Bypasses RNA Extraction. *bioRxiv*, June 18, 2020, ver. 1. DOI: 10.1101/2020.06.18.159434 (accessed Feb 15, 2021).

(48) Vogels, C. B. F.; Watkins, A. E.; Harden, C. A.; Brackney, D. E.; Shafer, J.; Wang, J.; Caraballo, C.; Kalinich, C. C.; Ott, I. M.; Fauver, J. R.; Kudo, E.; Lu, P.; Venkataraman, A.; Tokuyama, M.; Moore, A. J.; Muenker, M. C.; Casanovas-Massana, A.; Fournier, J.; Bermejo, S.; Campbell, M.; et al. SalivaDirect: A Simplified and Flexible Platform to EnhanceSARS-CoV-2 Testing Capacity. *Med.* **2021**, *2*, 263−280.e6.

(49) Wang, H.; Liu, Q.; Hu, J.; Zhou, M.; Yu, M.-q.; Li, K.-y.; Xu, D.; Xiao, Y.; Yang, J.-y.; Lu, Y.-j.; Wang, F.; Yin, P.; Xu, S.-y. Nasopharyngeal Swabs Are More Sensitive Than Oropharyngeal Swabs for COVID-19 Diagnosis and Monitoring the SARS-CoV-2 Load. *Front. Med.* **2020**, *7*, 334.

(50) Biden, President Joseph R., Jr. *National Strategy for the COVID-19 Response and Pandemic Preparedness*. https://www.whitehouse.gov/wp-content/uploads/2021/01/National-Strategy-for-the-COVID-19-Response-and-Pandemic-Preparedness.pdf (accessed 2021-04-05).

(51) *Ellume Awarded $231.8M From US DoD, HHS for OTC, At-Home COVID-19 Test*. https://www.360dx.com/immunoassays/ellume-awarded-2318m-us-dod-hhs-otc-home-covid-19-test#.YB2n6HdkgRU%20 (accessed 2021-04-05).

(52) National Institutes of Health. *NIH Mobilizes National Innovation Initiative for COVID-19 Diagnostics*. https://www.nih.gov/news-events/news-releases/nih-mobilizes-national-innovation-initiative-covid-19-diagnostics (accessed 2021-04-06).

(53) *NIH RADx Program Awards $29.3M to Increase SARS-CoV-2 Testing Capacity*. https://www.360dx.com/covid-19/nih-radx-program-awards-293m-increase-sars-cov-2-testing-capacity?utm_source=Sailthru&utm_medium=email&utm_campaign=360DN%20Tues%202021-04-06&utm_term=360Dx%20Daily%20News#.YGx5umRKjeo (accessed 2021-04-06).

(54) Payne, S. Methods to Study Viruses. In *Viruses From Understanding to Investigation*; Academic Press: London, 2017.

(55) Korber, B.; Fischer, W. M.; Gnanakaran, S.; Yoon, H.; Theiler, J.; Abfalterer, W.; Hengartner, N.; Giorgi, E. E.; Bhattacharya, T.; Foley, B.; Hastie, K. M.; Parker, M. D.; Partridge, D. G.; Evans, C. M.; Freeman, T. M.; Silva, T. I. d.; et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell* **2020**, *182*, 812−827.e19.

(56) Harrison, A. G.; Lin, T.; Wang, P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends Immunol.* **2020**, *41*, 1100−1115.

(57) Tang, H.; Abouleila, Y.; Si, L.; Ortega-Prieto, A. M.; Mummery, C. L.; Ingber, D. E.; Mashaghi, A. Human Organs-on-Chips for Virology. *Trends Microbiol.* **2020**, *28*, 934−946.

(58) Shpichka, A.; Bikmulina, P.; Peshkova, M.; Kosheleva, N.; Zurina, I.; Zahmatkesh, E.; Khoshdel-Rad, N.; Lipina, M.; Golubeva, E.; Butnaru, D.; Svistunov, A.; Vosough, M.; Timashev, P. Engineering a Model to Study Viral Infections: Bioprinting, Microfluidics, and Organoids to Defeat Coronavirus Disease 2019 (COVID-19). *Int. J. Bioprint.* **2020**, *6*, 302.

(59) Centers for Disease Control and Prevention. *Frequently Asked Questions about COVID-19 Vaccination*. https://www.cdc.gov/coronavirus/2019-ncov/vaccines/faq.html (accessed 2021-02-11).

(60) Isho, B.; Abe, K. T.; Zuo, M.; Jamal, A. J.; Rathod, B.; Wang, J. H.; Li, Z.; Chao, G.; Rojas, O. L.; Bang, Y. M.; Pu, A.; Christie-Holmes, N.; Gervais, C.; Ceccarelli, D.; Samavarchi-Tehrani, P.; Guvenc, F.; Budylowski, P.; Li, A.; Paterson, A.; Yun, Y. F.; et al. Persistence of Serum and Saliva Antibody Responses to SARS-CoV-2 Spike Antigens in COVID-19 Patients. *Sci. Immunol.* **2020**, *5*, eabe5511.

(61) Maple, P. A. C.; Sikora, K. How Useful is COVID-19 Antibody Testing: A Current Assessment for Oncologists. *Clin. Oncol.* **2021**, *33*, e73−e81.

(62) Price-Haywood, E. G.; Burton, J.; Fort, D.; Seoane, L. Hospitalization and Mortality among Black Patients and White Patients with COVID-19. *N. Engl. J. Med.* **2020**, *382*, 2534−2543.

(63) Centers for Disease Control and Prevention. *Health Equity Considerations and Racial and Ethnic Minority Groups*. https://www.cdc.gov/coronavirus/2019-ncov/community/health-equity/race-ethnicity.html (accessed 2021-04-06).

https://doi.org/10.1021/acsnano.1c02981
ACS Nano XXXX, XXX, XXX−XXX